bio-protocol Preprint

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Plaque assays and immunostaining.

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An abbreviated version of this protocol was published in Journal of Virology in Mar 2021

Generation and Characterization of Recombinant SARS-CoV-2 Expressing Reporter Genes

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Detailed protocol

Plaque assay and immunostaining protocol:

1. Prepare Vero E6 cells in 6-well plates (~1x10^6 cells/well) the day before plaque assay in cell culture media (DMEM+10%FBS+1%PSG).
2. Dilute the virus-containing cell culture supernatant using 10-fold serial dilutions in post-infection medium (DMEM+2%FBS+1%PSG).
3. Wash cells 2 times with DMEM+2%FBS+1%PSG and infect them with the diluted supernatants. Incubate for 1 h at 37°C.
4. During viral infection, prepare the semi-solid medium (0.6% agar): 2x DMEM-F12, 25 ml; cell culture water, 9.5 ml; 1% DEAE-Dextran, 0.5 ml; and 2% Agar, 15 ml.
5. After 1 h infection, discard the infection media and wash cells with DMEM+2%FBS+1%PSG.
6. Overlay the cells with 4 mL of semi-solid medium. Let solidify the semi-solid medium by leaving the plates at room temperature for ~10 min.
7. Incubate the plates invertedly at 37°C in a 5% CO_2 incubator for 72 h.
8. After 72 h viral infection, fix the plates by submerging in 10% formalin overnight. After overnight fixation in 10% formalin, plates can be moved from the BSL3 to the BSL2 lab, as determined by institutional biosafety policies.
9. Remove the semi-solid medium from the 6-well plates and permeabilize the cells with 2 ml of 0.5% Triton X-100 at room temperature for 10 min.
10. Wash the cells 3 times with PBS.
11. Block the cells with 1 mL/well of 2.5% BSA solution at 37°C for 30 min.
12. Wash the cells 3 times with PBS.
13. Dilute the primary monoclonal antibody against the viral N protein (1C7C7) in 2.5% BSA solution to a final concentration of 1 µg/mL and incubate with the cells with 1 mL/well at 37°C for 1 h.
14. Wash the cells 3 times with PBS.
15. Dilute the biotinylated horse anti-mouse secondary antibody in 2.5% BSA solution, as recommended by the company, and incubate with the cells with 1 mL/well at 37°C for 1 h.
16. Prepare the ABC reagent by mixing 100 µL Reagent A and 100 µL Reagent B in 10 mL of PBS and incubate at 37°C for 30 min.
17. Wash the cells 3 times with PBS.
18. Incubate the cells with the ABC reagent (1 mL/well) at 37°C for 30 min.
19. Wash the cells 3 times with PBS.
20. Prepare DAB substrate by mixing 2 drops of Reagent 1, 4 drops of Reagent 2, 2 drops of Reagent 3, and 2 drops of Reagent 4 in 5 mL deionized water.
21. Visualize the plaques by overlaying 1 mL of the prepared DAB substrate onto the cells immediately.
22. Stop the staining by washing the cells with PBS after discarding the substrate solution.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Ye, C. and Martinez-Sobrido, L. (2022). Plaque assays and immunostaining.. Bio-protocol Preprint. bio-protocol.org/prep1859.
2. Chiem, K., Vasquez, D. M., Park, J., Platt, R. N., Anderson, T., Walter, M. R., Kobie, J. J., Ye, C. and Martinez-Sobrido, L.(2021). Generation and Characterization of Recombinant SARS-CoV-2 Expressing Reporter Genes. Journal of Virology 95(7). DOI: 10.1128/JVI.02209-20